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MOLECULAR DOCKING STUDIES TO DESIGN POTENTIAL ANTIMALARIAL COMPOUNDS TARGETING RAB-6

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Abstract

Malaria is an ancient disease which still causes more than two hundred million of cases with high mortality globally. Identification of new drug targets as well as development of novel antimalarial drugs with unique mode of action can encounter the drug resistance and reduce the mortality by *Plasmodium* parasites. Ras-related protein Rab-6 is one of the key proteins in *P. falciparum* playing important roles including cellular proliferation, differentiation, survival and gene expression. Ras-related protein Rab-6 show significant structural deviations in specific regions involved in binding of regulatory proteins and can provide opportunities for structure-based drug design. Due to structural deviation the binding cavity of Ras-related protein Rab-6 it is suggested as a drug target for structural based drug designing. Structural based molecular docking approach was developed and used to screen potential lead molecules from the original molecular library of diverse natural molecules against Ras-related protein Rab-6. The top scoring molecules were consider potential hits as they display high binding scores and also good interaction pattern were also observed with Ras-related protein Rab-6. In general, this study provides a set of lead molecules which can be further explored through in *vitro* and *in vivo* experiments for development of potential drugs against malaria, which can encounter drug resistance and establish Ras-related protein Rab-6 as an important drug target.

Key words: Molecular docking, malaria, Rab-6, drug, *P. falciparum*.

Introduction

Malaria is a mosquito-borne infectious disease that infects humans and other animals. It is caused by five species of genus *Plasmodium*, which are transmitted through the bite of female *Anopheles* mosquito. Malaria, causes symptoms i.e. fever, headache and vomiting. In 2017 as per WHO report, 219 million cases of malaria were reported in 87 countries and the estimated number of deaths was found to be 435,000. Although there is still no widely used effective vaccine available for malaria parasites (Knox and Redmond, 2006). The most popular antimalarial drugs were used against malarial parasites *i.e.* Chloroquine, Chloroguanide (Proguanil), Sulfadoxine/ pyrimethamine (SP), Quinine, Mefloquine, Halofantrine and Atovaquone.

Both drug resistance and unavailability of highly effective vaccine makes malaria a highly challenging disease and a great public health burden. Identification of new/novel drug targets as well as development of novel antimalarial drugs with unique mode of action can be the possible answer to drug resistance in malaria and subsequently it can control the mortality due to malaria (White, 2004). Being highly studied diseases several potential drug targets have been proposed in research which can be additionally evolved as a drug target in experiments. Ras-related protein Rab-6 was also reported in the literature and can have a potential to develop vaccine candidate or drug target in different pathogen diseases. Recently, our group has discovered that Ras-related protein is expressed in almost all stages of *Plasmodium falciparum* present in human which justifies its importance in plasmodium life cycle and its drug target potential (Guleria and Jaiswal, 2020). Ras-related protein Rab-6 is not yet studied for structure based

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drug design and consequently not developed as an important drug target (Guleria and Jaiswal, 2020). But in different literature this protein is planned as the drug target in malaria. In different stages of *Plasmodium* Ras-related protein Rab-6 is novel drug target and can be a possible answer to drug resistance (Guleria and Jaiswal, 2020). Although this protein is conserved in both but there is difference between human and *Plasmodium.* Ras-related protein rab was potential vaccine candidate against *cutaneous* and *visceral* leishmaniasis. It plays an essential role in the regulation of the secretary pathway in *Leishmania* (Bahl, Parashar *et al*., 2015). Ras-related protein is a member of superfamily of small GTPases. It can be separated into three subgroups *i.e*. Rab subgroup, Rac subgroup and Rho subgroup (McCormick, 1995). The Rab subgroup protein complicated in membrane trafficking and secretion. The Rac and Rho subgroup contains proteins both are complex in organizing diverse aspects of the cytoskeleton. The Ras-related protein contains the H-, N- and K-Ras proteins (McCormick, 1995). Ras-related protein has two-domain one is a GTPase domain and second is C-terminal region. GTPase domain which binds GTP and GDP with very high similarity. RAS proteins are dual molecular switches the cycle between active guanosine triphosphate (GTP)-bound and inactive guanosine diphosphate (GDP)-bound states (Simanshu, Nissley *et al*., 2017). The C-terminal domain must be process for the proteins to be inserting into the plasma membrane. In C-terminal signals two diverse are essential for specific plasma membrane localization *i.e*. farnesylation and palmitoylation (McCormick, 1995). Farnesylation modification which is essential for association of these proteins with membranes in common and palmitoylation requires a second set of modifications of H- and N-Ras with specific association of plasma membranes (McCormick, 1995). Ras-related proteins play a fundamental role in human cancer (Simanshu, Nissley *et al*., 2017). This protein is an essential intracellular signaling pathway that play important role in cellular proliferation, differentiation, survival and gene expression (Buday and Downward, 2008; Vigil, Cherfils *et al*., 2010). Ras oncoprotein has also been occupied in the improvement of cancer by enlarged intensity or prolonged signaling mechanism (Downward, 2003). With the size of ~21 kDa there are three human RAS genes encode four proteins i.e. H-Ras, N-Ras and the splice variants K-Ras4A and K-Ras4B. Ras-related protein is a soluble cytoplasmic protein, which requirements to endure post-translation modifications to associate with particular lipid membrane (Gurung and Bhattacharjee, 2015). These modifications occur at the carboxyl terminal 'CAAX' box (Gurung and Bhattacharjee, 2015). This protein consists of six β sheets and five α helices that are interrelated by a series of 10 loops. Out of ten loops five of loops find out the high-affinity nucleotide interactions of Ras and regulates GTPase activity (Roskoski Jr., 2010). In current years the Renin-Angiotensin system (RAS) has been involved in intra-host parasite interactions, polymorphisms in the genes and allied with protection against severe malaria (Silva, Silva-Filho *et al*., 2016). So, in current study Ras-related protein was taken for molecular docking studies because it is a new potential drug target and relatively not explored as drug target in drug discovery in spite of its conservation in different species of *Plasmodium*. Rab-6 *Plasmodium falciparum* was used to examine Golgi structural design in the malaria parasite. It is a small, GTP-binding protein that plays an important role in the instruction of vesicular trafficking in eukaryotic cells (Ward, Tilney *et al*., 1997). In parasite-specific Rab-6 *Plasmodium falciparum* antibodies were used in fluorescence microscopy assays and show a localisation distinctive from ERD2 in early stages (Van Wye, Ghori *et al*., 1996). Molecular docking is an important tool for drug discovery and can be used to model the interaction between small drug like molecule and a protein at atomic level, which permit us to describe the enactment of small molecules in the binding sites of target proteins as well as to irradiate the biochemical processes. It is very useful and one of the most usually used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. In the current study the library of small molecules is screened against Ras-related protein. The top scoring molecules from docking study were subjected for protein-ligand interaction studies. The best molecules according to these studies can be strongly recommended for further wet lab experiments of drug design.

Materials and Methods

Retrieval of protein and ligand libraries

The crystal structure of ras-related protein Rab-6 of *P. falciparum* (PDB ID: 1D57) was downloaded from protein databank in pdb format [https://www.rcsb.org/structure/1D57]. For virtual screening organic molecule NCI Diversity Set library consisting of total 1592 molecules was downloaded from zinc database in mol2 format.

Target protein preparation

The crystal structure of *P. falciparum* ras-related protein Rab-6 *(*PDB ID: 1D57) is of 2 $A^{R"}$ resolution with bound ligand GDP and have only one chains; chain A. It was observed that the binding pocket is also located in

Table 1: Binding affinity of selected potential molecules.

Zinc Id	Binding Affinity	Hydrogen Bonding	Hydrophobic Interactions
ZINC01755448	-10.68	Ser26	15
ZINC03954520	-9.43	Ser42	12
ZINC01566093	-9.28	Thr25,Lys24 & Lys125	9
ZINC00393856	-9.12	Ser26 & Lys125	12
ZINC13130018	-8.56	-	8
ZINC13152226	-8.49	-	12
ZINC17149568	-8.39	Gln20,Thr43,Thr25 & mg300	8
ZINC00258800	-8.31	Ser26,Ala155 & Lys156	11
ZINC01586128	-8.19	-	15
ZINC04783229	-7.94	Lys156 & Thr94	9

chain-A. The bound ligand molecule was also removed before docking. The protein was prepared for docking studies by assigning hydrogen, polaraties, calculating Gasteiger charges to protein structures and converting protein structures from the pdb format to pdbqt format using Auto-Dock tool 1.5.4.

Preparation of ligand libraries for virtual screening

NCI Diversity Set library was downloaded from ZINC database (https://zinc.docking.org/) in MOL2 format and was further used for performing docking studies. The library was in a single molecular file and all the ligand molecules were extracted from it with the help of in-house developed PERL scripts. Subsequently all the ligand molecules were converted into PDB format from MOL2 format by using open babel with the help of in-house developed PERL scripts.

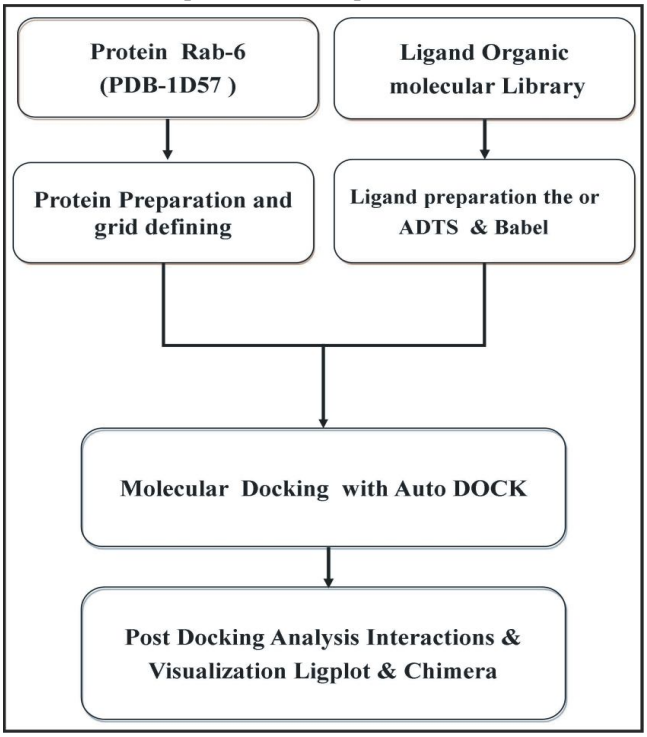


Fig. 1: The methodology followed for docking and screening.

Molecular docking

All the ligands within the organic molecular library were used to perform molecular docking studies against ras-related protein Rab-6 to find the potential lead molecules for further drug discovery experiments. In the current research for performing the docking studies the version 4.2 of AutoDock was used. AutoDock uses a Lamarckian Genetic Algorithm (LGA) and is based on a semi empirical free energy force field (Kalliokoski, Salo *et al*., 2009). The docking grid was set manually through visualization of the protein and grid was defined to cover entire binding pocket of ras-related protein Rab-6. Finally, molecular docking was carried out on target proteins with all ligands from the library using self-developed PERL script, which was used for screening of these large number of ligand molecules one by one (Fig. 1). The top scoring compounds were selected on the basis of binding energy of ligands with the receptor. The lower is the binding energy of ligand with the receptor the more strongly it will bound to the target receptor.

Interaction study and visualization of docked complex

The UCSF Chimera 1.8.1 was used to visualize and analyze docked complexes of ligand and protein. The interaction such as hydrogen bonds and hydrophobic interaction were also analyzed using Ligplot + (Kashyap, Jaiswal *et al*., 2017). The generated plots have shown the hydrophobic interaction patterns and hydrogen bonding between the main chain/side-chain atoms of the protein with the ligand.

Results

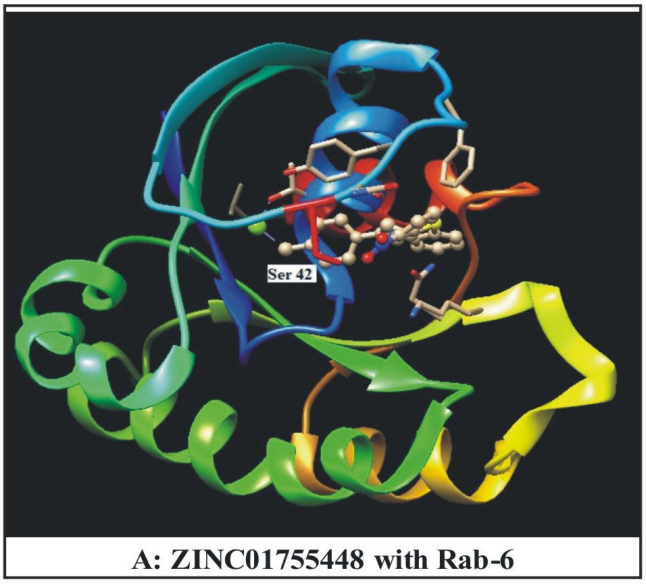
In the present study, considering the importance of ras-related protein Rab-6 protein as a drug target in *P. falciparum* molecular docking of ras-related protein Rab-6 of *P.falciparum* was carried out. The organic molecular library was docked into the active site of ras-related protein Rab-6 to find the potential ligand molecules which can be used in further drug design experiments and also establish the ras-related protein Rab-6 as an important drug target in malaria.

Docking analysis

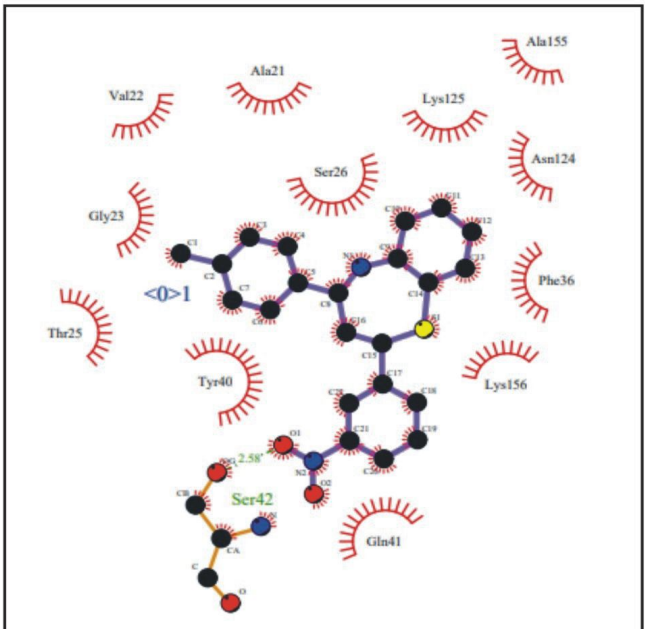
Molecular docking of all the ligands within the library was found to be successful on the basis that all the ligands docked in the active site of the receptor. The 10 top scoring molecules as per estimated free energy of binding were selected for further investigations. The protein ligand

Table 2: Physicochemical properties of potential compounds.

Zinc Id	Molecular Weight	Log P	Hydrogen Rotatable Bonds	Hydrogen Acceptors	Hydrogen Donors
ZINC01755448	398.458	5.56	4	4	0
ZINC03954520	374.465	6.26112	3	4	0
ZINC01566093	370.202	4.5833	2	3	1
ZINC00393856	311.768	4.75952	2	2	2
ZINC13130018	310.356	4.7371	0	3	2
ZINC13152226	295.345	4.76858	2	2	1
ZINC17149568	290.491	5.0261	0	1	1
ZINC00258800	265.287	4.2312	2	1	1
ZINC01586128	332.835	4.8129	3	3	2
ZINC04783229	365.789	4.921	4	5	3



A: ZINC01755448 with Rab-6



B: Interaction of ZINC01755448 with Rab-6

Fig. 2: Interactions of ZINC01755448 with Rab-6.

complexes of top scoring molecules were analyzed for interactions, the orientation of the docked compound and interacting active site residues were visualized. The selected 10 molecules had estimated free energy of binding in the range of -10.68 to -7.94 (Table 1). The molecule *i.e.* ZINC01755448 shows least estimated fee energy of binding of -10.68 (Table 1). The four other top scoring molecules ZINC03954520, ZINC01566093 and ZINC00393856 show the estimated fee energy of binding -9.43, -9.28, -9.12 respectively (Table 1). Although these molecules the other molecules also had estimated free energy of binding better than -8 and also show good interaction with the target molecule (Table 1) (Fig. 2 & 3). The top scoring molecule *i.e.* ZINC01755448 strongly interacts with the Ser26 residues of the binding cavity and showing strong hydrophobic interactions with 15 residues (Fig. 2) (Table 1). The second-best scoring molecule as per the estimated fee energy of binding *i.e.* ZINC03954520 forms hydrogen bonds with the Ser42 and also shows good hydrophobic interactions with 12 residues (Fig. 3) (Table 1).

The molecule ZINC01566093 strongly interacts with the Thr25, Lys24 & Lys125. All other selected molecules also show good patterns of hydrogen bond and hydrophobic interactions (Table 1).

Molecular parameters

Drug molecules having molecular weight less than 500 Da can easily transported, diffused and absorbed as that of the bigger molecules (Srimai, Ramesh *et al.*, 2013). The physicochemical properties were calculated and the molecular weight of all the selected 10 lead molecules was less than 500 Da. All the molecules (Table 2) showed logP value less than 5 (Table 2). The selected lead molecules have less than 10 hydrogen bond donors and also have less than 5 hydrogen bond acceptors (Table 2).

Discussion

Malaria is still a major global health problem, although antimalarial drugs are available but drug resistance has emerged thereby increasing morbidity and mortality rate are observed. The research for the development of efficient licensed malaria vaccine is still not over despite many rigorous efforts have been made in the last decade. It was previously suggested in the literature that the proteins such as actin, tubulin and histone are involved in the structural assembly of the pathogen (Samant, Chadha

et al., 2016). Hence, the potential new drug target ras-related protein Rab-6 is one of the most important structural proteins which are found in *P. falciparum* and plays important role was used to examine Golgi structural design in the malaria parasite and also plays role in the instruction of vesicular trafficking in eukaryotic cells (Ward, Tilney *et al*., 1997). It is expressed in all human stages and little is known about its potential as a drug target. In the current selection, we have consider library of 1592 NCI Diversity Set were docked with ras-related protein Rab-6 protein of *P. falciparum* employing one of the most important docking software (de Ruyck, Brysbaert *et al*., 2016) AutoDock 4.2. These approaches were widely utilized to design drugs against a number of pathologies such as cardiovascular diseases and cancer (Frédérick, Robert *et al*., 2005; Ahire, Das *et al*., 2016). The current study is the first report to explore the potential of receptor ras-related protein Rab-6 as an efficient drug target in structure-based drug design.

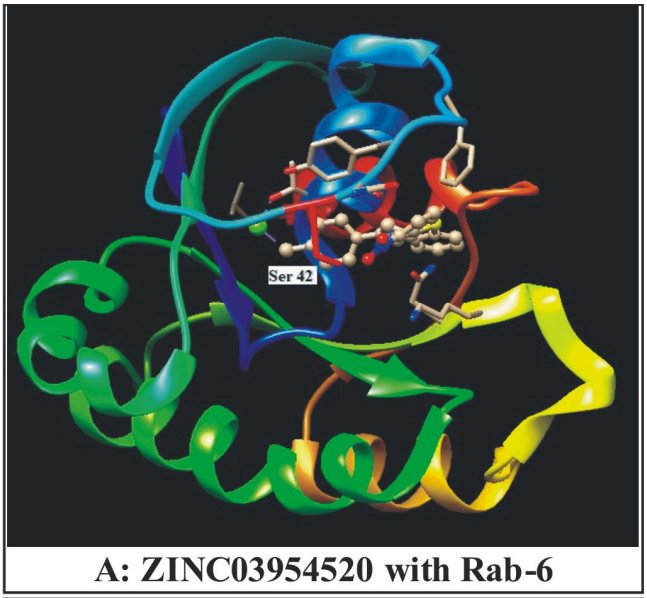


Fig. 3: Interaction of ZINC03954520 with Rab-6.

The prospect of virtual screening techniques was employed to screen the 10 top scoring molecules on the basis of their binding affinity. Although estimated free energy of binding (EFEB) of ligand protein interaction is relative score and direct correlation of this score is not studied in different docking studies but EFEB of selected ligands in current study was better than the ligands found to be active in recent *in silico* and *in vitro* studies conducted by our group in falcipains 2 and 3 of *Plasmodium* (Rana, Kalamuddin *et al*., 2020). Additionally, when the docked complexes of these selected lead molecules were subjected to post docking analysis, they displayed good interaction patterns (hydrogen as well as hydrophobic interactions). The compound with zinc ID ZINC01755448 was the top scoring molecule and also depicted good interactions indicating a potent promising candidate. Similar, affinity was also discovered for other remaining 9 molecules which were also prioritized as lead molecules displaying good binding affinities and interaction patterns (Table 1). Also, these top scoring molecules interact strongly with the target protein and forming a number of hydrogen bonds (Table 1) (Fig. 2 and 3). In most of these top scoring molecules Ser26, Ser42 & Lys125 were found to be most common interacting residues forming hydrogen bonding.

The molecular weight and logP was observed to be slightly higher as per the standard rules, but natural origin and all other parameters justified that the above potential lead molecule can be developed against malaria which can serve as a roadmap for perusing drug discovery process. The remaining molecules having zinc ID ZINC00258800, ZINC01586128 and ZINC04783229 also showed high binding affinities and these molecules also justified the different essential parameters to be considered as lead molecules for the drug discovery (Table 1) (Fig. 2 & 3). Moreover, these selected lead molecules can be subjected to further wet lab experimentations and consequently modification therefore so that these can be used as potential lead molecules for drug discovery.

Conclusion

First time virtual screening methodology was

implemented using diverse organic molecular library to find potential lead molecules for ras-related protein Rab-6 of *P. falciparum* in structure-based drug design. Docking score, interaction of top selected scoring molecules were found to be favorable to pursue further drug development with these molecules. The current findings provide a suitable starting point for further *in vitro* and *in vivo* analyses to exploit ras-related protein Rab-6 as an optimal drug target which can encounter drug resistance.

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